INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK
WAGENINGEN, NEDERLAND
DIRECTEUR: Dr. J. G. TEN HOUTEN

MEDEDELINGEN No 178 EN 179

INVESTIGATIONS ON A LEAFHOPPER-BORNE CLOVER VIRUS

DOOR
H. H. EVENHUIS

DE VECTOREN VAN HET BLOEMVERGROENINGSVIRUS VAN KLAVER

(THE VECTORS OF THE VIRUS CAUSING PHYLLODY (VIRESCENCE) IN CLOVER FLOWERS)

DOOR
H. H. EVENHUIS

Dere with conference Per.

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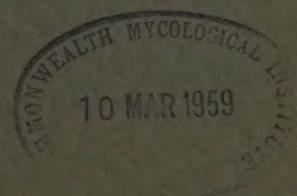

INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK (I.P.O.)

Office and main laboratory: Binnenhaven 4a, tel. 2151, 2152 en 3641 Wageningen, The Netherlands.

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Drs. J. C. ZADOKS, Phytopathologist

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Drs. L. E. VAN 'T SANT, Entomologist } in de volle grond", Alkmaar, tel. K 2200-4568.
Drs. D. J. DE JONG, Entomologist } detached to „Proefstation voor de Fruitteelt in de
Ir. G. S. ROOSJE, Phytopathologist } volle grond", Wilhelminadorp, Goes, tel. K 1100-2261
Ir. T. W. LEFERING, Phytopathologist/Virologist, detached to „Proeftuin Noord Limburg" Venlo, tel. K 4700–2503.
Drs. G. SCHOLTEN, Phytopathologist, detached to „Proefstation voor de bloemisterij in Nederland", Aalsmeer, tel. K 2977–688.
Ir. G. P. TERMOHLEN, Phytopathologist, detached to „Proeftuin voor de Groente- en Fruitteelt onder glas", Naaldwijk, tel. K 1740–4545.

Guest workers:

Dr. P. A. VAN DER LAAN, Entomologist, „Laboratorium voor toegepaste Entomologie der Gemeente Universiteit," Amsterdam, tel. K 2900–56282.
Dr. Ir. G. S. VAN MARLE, Entomologist, Diepenveenseweg 226, Deventer. tel. K 6700–3617.
Ir. G. W. ANKERSMIT, Entomologist, „Laboratorium voor Entomologie", Agricultural University, Wageningen, tel. K 8370–2438.
Dr. Ir. J. B. M. VAN DINTHER, Entomologist, „Laboratorium voor Entomologie", Agricultural University, Wageningen, tel. K 8370-2438.

Aphidological Adviser:

Mr. D. HILLE RIS LAMBERS, Entomologist, T.N.O., Bennekom, tel. K 8379–458.

INVESTIGATIONS ON A LEAFHOPPER-BORNE CLOVER VIRUS

H. H. EVENHUIS

Institute of Phytopathological Research, Wageningen, the Netherlands

Some years ago MARAMOROSCH (1953) described a new leafhopper-borne virus disease from the Netherlands. He collected some leafhopper species in the vegetation of a cherry orchard at Gronsveld in the neighbourhood of Maastricht and transferred them in the laboratory at Wageningen to different test plants.

Several specimens of *Euscelis plebejus*, if transferred to crimson clover seedlings, caused symptoms, which MARAMOROSCH attributed to a virus disease, new for Western Europe. He described two groups of symptoms: 1. the first signs, which were characterized by enlarged veins and deep ridges on the upper surface of the leaflets, often with pronounced enations on the lower surface on the veins; 2. the later symptoms, a chlorosis which progressed from the outer edges towards the midribs of the leaflets and small malformed leaves on shortened petioles.

We did some experiments in the summer of 1953 in order to get more information about the supposed virus of MARAMOROSCH.

On each of 35 crimson clover plants two to ten specimens of *Euscelis plebejus* were caged for a week. The insects were obtained from the same orchard where MARAMOROSCH collected his leafhoppers. After some time eight plants showed only the first named group of symptoms, three only the second group and three both groups of symptoms. These data show, that the two groups of symptoms may appear separately and that there is no relation between these two groups of symptoms. In the following the two groups of symptoms will be considered separately.

1. The enlarged veins, the deep ridges and the enations may already appear after a rather short time of feeding of the insect; according to MARAMOROSCH in some cases the symptoms already appeared within a week. We noticed the symptoms after a somewhat longer interval, but in most cases they were very conspicuous after two weeks. On some plants the symptoms were much more pronounced than on others; also it was noted that the same leafhoppers sometimes did produce the symptoms on one plant but not on another. Besides, no correlation could be found between the number, the size of the leafhoppers in the different instars or the size of the crimson clover plants and the intensity of the symptoms. Sometimes only one small nymph already sufficed to produce very severe symptoms. In the winter season there were mostly no symptoms at all.

The symptoms could also be produced on *Trifolium hybridum* and exceptionally also on white clover. No symptoms were obtained on red clover, pea, bean, alfalfa and white sweet clover. All further experiments have been done with crimson clover.

Besides *Euscelis plebejus* also some other leafhopper species have been tested. The taxonomy of the genus *Euscelis* is very difficult, but the work of WAGNER, RIBAUT and especially the recent biological studies of MÜLLER (1954, 1955) have contributed considerably to our knowledge on this subject. The taxonomy has not yet been entirely elucidated, but it appears that practically all forms, found regulary in the grass, belong to the species *E. plebejus* and *E. lineolatus* and both species are equally capable of

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producing the symptoms on crimson clover. Both species are very polyphagous and may breed on widely different plant species.

No symptoms were produced on crimson clover by *Aphrodes bicinctus, Macrosteles spec.* and *Arthaldeus pascuellus*. The latter is a grass feeding species that cannot live for a long time on crimson clover.

Since symptoms as the above mentioned can be caused by a virus as well as by a toxic substance, the nature of the causative agent should be determined experimentally. If it would be possible to rear a strain of *Euscelis*, which does not produce symptoms on healthy clover plants until after a period of feeding on diseased plants, proof of the virus nature of the agent would be delivered.

Attempts to produce such a strain have, however, not been successful, not even if insects were hatched from isolated eggs between wet filterpaper in Petri dishes. Nymphs reared this way were capable of producing the symptoms on healthy plants. From this fact we must conclude that if a virus is concerned it must have been transmitted easily through the egg.

An argument against the virus nature of the agent, however, is the fact that if a leafhopper is put on a healthy plant, the symptoms occur in the leaves unfolding subsequently, but not in those unfolding some time after the insect has been removed from the plant. For instance: on a plant on which five leafhoppers had been feeding from September 11 to 14, distinct symptoms appeared only on one leaf on September 26, the younger leaves showing no symptoms at all. On another plant a nymph had been feeding from May 28 till June 24. Distinct symptoms appeared between June 17 and July 15 on seven leaves, whereas on the younger leaves no symptoms appeared.

If one puts a little cage with a leafhopper round a leafstalk, the new, unfolding leaves show the symptoms. This implies that the symptoms are induced by a toxic substance that spreads through the plant, through the leaf stalk to the vegetation point.

In literature more cases are known of leafhoppers producing viruslike symptoms, caused by a systemic toxic substance. KUNKEL (1933) describes a wilting of young peach seedlings caused by *Macropsis trimaculata*; SEVERIN, DOUGLAS HORN & FRAZIER (1945) describe viruslike symptoms on sugar beet and aster induced by the feeding of the leafhopper *Xerophloea vanduzeei*; SEVERIN (1947) in California mentions the appearance of symptoms by feeding of specimens of 10 leafhopper vectors of aster yellows on aster and celery; BENNETT (1952) reports a veinclearing on sugar beet caused by a systemic toxic substance, introduced into the plant by the feeding of some specimens of *Circulifer tenellus*.

2. The second group of symptoms, described by MARAMOROSCH, may appear after a much longer period; in most cases not until after five or six weeks. From 1953 until 1956 we were not able to reproduce these symptoms. However, in 1956 we found at Gronsveld, near Maastricht, a red clover plant with viruslike symptoms. These symptoms appeared to be produced by a virus, that was transmitted by the leafhoppers *Euscelis plebejus* and *E. lineolatus* from diseased plants to healthy red clover seedlings. Leafhoppers, reared in cages on healthy seedlings, did not produce these symptoms.

The symptoms were a distinct vein-chlorosis or vein-clearing on the leaves, which seemed darker than the healthy ones; later on a stunting and proliferation of the plant, a yellowing of the young leaves beginning at the edges, a production of very small leaves and a phyllody of the flowers appeared. The symptoms proved to be identical with those, described by FRAZIER & POSNETTE (1956), who were the first to prove that this disease is really a virus disease, transmitted by leafhoppers.

The virus was also transmitted by *Euscelis plebejus* and *E. lineolatus* to crimson clover, producing the same symptoms. So we arrived at the conclusion that the second group of symptoms on crimson clover, described by MARAMOROSCH, are produced by the same virus. However, in 1953 a phyllody of the flowers was never observed, because of the fact that the plants with virus symptoms did not flower at all. We were also able to prove, that *Aphrodes bicinctus* is capable to transmit the virus. In the meantime FRAZIER & POSNETTE (1957) found that the virus they described in 1956 is in fact a complex of two viruses of which one only produces the phyllody and the other especially the symptoms of witches' broom.

Up till now we were not able to separate the two viruses, so when speaking about the clover virus it may be possible that two viruses are concerned.

We were able to transmit the virus by means of *Euscelis spec.* in one of four cases to aster. On this aster plant we got a slight chlorosis of the leaves, but a very pronounced phyllody with green, rather small flower heads and especially phyllody of the ovary, just as in clover. From the symptoms on aster we conclude, that the virus disease of clover is not identical with the european aster yellows, described by HEINZE & KUNZE (1955), nor with the American aster yellows (eastern and western strain).

The symptoms, shown by red and crimson clover, were also obtained on white clover. By feeding virus-infected leafhoppers on peas also a phyllody was obtained. We also used *Cuscuta subinclusa* in our experiments; by means of this dodder species the virus could easily be transmitted from red clover to red clover.

No exact data are known about the period, necessary for the leafhoppers to pick up the virus from a diseased plant and to transmit it to a healthy plant. At any rate four days feeding on a diseased plant and three days feeding on a healthy plant are sufficient. Probably much shorter times would also be sufficient to obtain virus transmission.

The latent period in the vector is very long. By transferring every week leafhoppers that had been feeding on a diseased plant to a new healthy seedling of red clover in the glasshouse, the latent period in the vector was estimated to amount six to seven weeks or even more.

The nymphs as well as the adults can pick up the virus. As the latent period lasts very long, the nymphs had become adults when the insects became infective.

Phyllody of clover in Western Europe is already known for a very long time. It was already mentioned in the handbook of MASTERS (1869). Also PENZIG (1890) states, that phyllody of white clover is very common. In earlier times it was ascribed to several different causes, which BECKER (1941) mentions in his paper. FRAZIER & POSNETTE (1956) were the first to prove that a phyllody of clover is caused by a virus, transmitted by leafhoppers. They were able to transmit the virus by dodder to strawberries on which the typical symptoms of the green petal disease of strawberries developed.

So the symptoms, described by MARAMOROSCH from crimson clover and caused by feeding of the leafhopper *Euscelis spec.* may be divided in two distinct groups: 1. symptoms which are not caused by a virus, but by a toxic substance, spreading systemically through the plant; 2. symptoms, caused by a virus, identical with the virus, described by FRAZIER & POSNETTE. It can be transmitted by some leafhopper species to a number of plant species.

The first group of symptoms have only been observed, after *Euscelis spec.* had been feeding on crimson clover, on *Trifolium hybridum* and in one case on white clover.

SUMMARY

A further study was made of the leafhopper-borne disease of crimson clover described from the Netherlands by MARAMOROSCH. The first symptoms, principally consisting of deep ridges on the upper side of the leaflets, proved to be caused by a toxic substance excreted by the leafhopper. These symptoms appeared rather shortly (sometimes already after one week) after the leafhoppers had fed on the plant. The later symptoms which were among others a chlorosis, progressing from the margins towards the midribs of the leaflets, appeared after a much longer period (minimum: 3 to 4 weeks). These symptoms proved to be caused by a virus, transmitted by *Euscelis plebejus*, *E. lineolatus*, and also by *Aphrodes bicinctus*. The virus involved is probably the same as the one causing green petal disease of strawberry, described by FRAZIER & POSNETTE. Judged by the symptoms produced in aster the virus is not identical with the virus causing European aster yellows, described by HEINZE & KUNZE, or with the eastern and western strains of aster yellows in the United States of America.

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DE VECTOREN VAN HET BLOEMVERGROENINGSVIRUS VAN KLAVER[1])

With a summary: The vectors of the virus causing phyllody (virescence) in clover flowers

DOOR

H. H. EVENHUIS

Instituut voor Plantenziektenkundig Onderzoek, Wageningen

Bloemvergroeningsverschijnselen zijn al minstens sedert de vorige eeuw uit West-Europa bekend (BOS, 1957). Door FRAZIER & POSNETTE (1956) is voor het eerst aangetoond, dat deze verschijnselen kunnen worden veroorzaakt door een virus, dat door cicaden wordt overgebracht. In 1957 vermeldden de beide genoemde auteurs, dat het bewuste virus kan worden gesplitst in twee componenten, die in verwekte symptomen verschillen, doch niet in de manier van overbrengen. De scheiding in twee componenten is ons tot op heden nog niet gelukt; daarom zullen we hier blijven spreken van één virus en wel van het bloemvergroeningsvirus.

Dit virus blijkt allerminst specifiek te zijn voor één bepaalde plantesoort; FRAZIER & POSNETTE (1956, 1957) en EVENHUIS (1957) vermelden een groot aantal waardplanten, die tot zeer verschillende families behoren. Er blijken eveneens verschillende vectoren te bestaan. FRAZIER & POSNETTE (1956, 1957) vermelden *Euscelis lineolatus* BRULLÉ, *E. plebejus* (FALL.) en *Macrosteles viridigriseus* (EDWARDS) als zodanig, BOVEY (1957) en EVENHUIS (1957) ook nog *Aphrodes bicinctus* (SCHRANK).

In 1957 werden nog enkele overbrengingsproeven met verschillende andere soorten cicaden verricht. Zo werd met *Philaenus spumarius* (L.) en *Agallia consobrina* (CURT.), die beide polyfaag zijn, een groot aantal proeven opgezet; in geen enkel geval werd echter overbrenging van het virus verkregen. Wel bleek het virus te kunnen worden overgebracht door *Aphrodes albifrons* (L.) (in twee gevallen) en *Macrosteles cristatus* (RIBAUT) (in één geval). Dat er slechts weinig gevallen van overbrenging met deze beide soorten werden verkregen, is het gevolg van het nog geringe aantal proeven, dat met deze soorten werd uitgevoerd. Bovendien bedraagt de duur van de latente periode van het virus in het insekt in de proeven in de warme kas meestal zes tot zeven weken en is het vaak moeilijk – vooral bij de tere soorten – om de dieren zo lang in leven te houden. De kans is groot, dat bij verder onderzoek zal blijken, dat nog meer soorten cicaden het bloemvergroeningsvirus kunnen overbrengen; vooral in de geslachten *Macrosteles* en *Aphrodes* is deze kans groot. Op het ogenblik zijn er dus zes verschillende vectoren van het virus bekend.

Niet alle vectoren zijn even effectief, zo blijkt *Aphrodes bicinctus* een veel minder effectieve overbrenger te zijn dan *Euscelis plebejus* en *E. lineolatus*. Trouwens ook alle waardplanten zijn niet even geschikt; zo gelukte de overbrenging van het virus op klaver (witte, rode en inkarnaatklaver) veel beter dan op aster.

[1]) Aangenomen voor publikatie 20 mei 1958.

SUMMARY

In literature the leafhopper species *Euscelis plebejus* (FALL.), *E. lineolatus* BRULLÉ, *Macrosteles viridigriseus* (EDWARDS) and *Aphrodes bicinctus* (SCHRANK) are mentioned as vectors of the virus causing phyllody in clover and other flowers. It was also found that *Macrosteles cristatus* (RIBAUT) and *Aphrodes albifrons* (L.) are able to transmit the virus. It seems probable that more vectors of the virus exist. However in many experiments with *Philaenus spumarius* (L.) and *Agallia consobrina* (CURT.) no transmission of the virus was obtained.

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